

Dynamics of Photosystem Stoichiometry Adjustment by Light Quality in Chloroplasts¹

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Long-term imbalance in light absorption and electron transport by photosystem I (PSI) and photosystem II (PSII) in chloroplasts brings about changes in the composition, structure, and function of thylakoid membranes. The response entails adjustment in the photosystem ratio, which is optimized to help the plant retain a high quantum efficiency of photosynthesis (W.S. Chow, A. Melis, J.M. Anderson [1990] *Proc Nat Acad Sci USA* 87: 7502–7506). The dynamics of photosystem ratio adjustment were investigated upon the transfer of pea (*Pisum sativum*) plants from a predominantly PSI-light to a predominantly PSII-light environment and vice versa. The concentration of functional components (primary electron accepting plastoquinone of PSII [Q_A], P700) and that of constituent proteins were monitored during acclimation by a difference spectrophotometry and immunoblot analysis, respectively. Fully reversible changes in photosystem ratio occurred with a half-time of about 20 h. They involved closely coordinated changes in the concentration of the Q_A, reaction center protein D1, D2, and the 9-kD apoprotein of the cytochrome *b*₅₅₉ for PSII. Similarly, closely coordinated changes in the relative concentration of P700 and reaction center proteins of PSI were observed. The level of chlorophyll *b* and that of the light-harvesting complex II changed in accordance with the concentration of PSII in the acclimating thylakoids. Overall, adjustments in the photosystem ratio in response to PSI- or PSII-light conditions appeared to be a well-coordinated reaction in the chloroplast. The response was absent in the chlorophyll *b*-less chlorina f2 mutant of barley (*Hordeum vulgare*) and in a phycobilisomeless mutant of *Agmenellum quadruplicatum*, suggesting that photosystem accessory pigments act as the light-quality perception molecules and that PSI and PSII themselves play a role in the signal transduction pathway.

Under limiting intensity of illumination, the efficiency of photosynthesis depends on the coordinated interaction of two photosystems in the electron-transport chain. PSII is involved in the oxidation of water and reduction of plastoquinone, whereas PSI enables electron transport from plastoquinone and from the Cyt *b*-*f* complex to Fd. The quantum yield of photosynthesis in many plant species from diverse light habitats is about 0.106 ± 0.001 mol of O₂ evolved mol⁻¹ of photons absorbed (Ley and Mauzerall, 1982; Björkman and Demmig, 1987; Evans, 1987). This value is very close to a theoretical upper limit of 0.125 mol of O₂ evolved mol⁻¹ of photons absorbed, translating in a quantum efficiency of about 85%, independent of the light climate in

which plants grow. This is a remarkable feature of the photosynthetic apparatus, given the contrasting light qualities that prevail in different plant ecosystems (Björkman and Ludlow, 1972; Kirk, 1983; Terashima and Saeki, 1983) and the fact that substantially different pigments absorb light for PSI and PSII in the thylakoid membrane of oxygenic photosynthesis.

It is known that strong gradients in light quality occur within a single leaf (Terashima and Saeki, 1983; Vogelmann, 1989), within the canopy of a single tree, or within the canopy of a forest (Björkman and Ludlow, 1972) and within the aquatic environment (Kirk, 1983). Some of these environments favor absorption of light by PSI; others favor absorption of light by PSII (Glazer and Melis, 1987). Thus, a question was raised as to how plants may perform photosynthesis with maximal efficiency under a variety of contrasting light qualities. Recent work (Murakami and Fujita, 1988; Melis et al., 1989; Chow et al., 1990) provided direct evidence that adjustment of the photosystem ratio in thylakoids may be a key to the high quantum efficiency of photosynthesis under diverse light-quality conditions. The mechanism for the adjustment of the photosystem ratio appears to be highly conserved in nature, because O₂-evolving organisms from cyanobacteria to higher plants are known to possess it (Kawamura et al., 1979; Myers et al., 1980; Melis and Harvey, 1981; Wilhelm and Wild, 1984; Glick et al., 1985; Wilhelm et al., 1985; Glick et al., 1986; Fujita et al., 1987; Murakami and Fujita, 1988; Deng et al., 1989; Cunningham et al., 1990). It was inferred that such a mechanism performs a highly needed function in O₂-evolving plants (Chow et al., 1990).

The dynamic response of thylakoid membranes and the adjustment of photosystem stoichiometry to different light-quality conditions suggested the existence of a mechanism capable of recognizing imbalance in the rate of light utilization by the two photoreactions and directing cellular metabolic activity for photosystem stoichiometry adjustments (Melis, 1991). Clearly, the acclimation mechanism confers to plants a significant evolutionary advantage over that of a fixed photosystem ratio in thylakoid membranes (Chow et al., 1990). The notion of a "flexible" or "dynamic" thylakoid membrane of photosynthesis is accepted by most investigators in the field (Björkman et al., 1972; Lichtenthaler and Meier, 1984; Anderson, 1986; Leong and Anderson, 1986; Glazer and Melis, 1987; Chow et al., 1989; Jursinic and Dennenberg, 1989; Melis, 1991). However, the underlying

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Abbreviations: LHC-II, light-harvesting complex of PSII; PBS, phycobilisome; Q_A, primary electron accepting plastoquinone of PSII.

molecular mechanism for the control of the PSII:PSI ratio and the regulation of biosynthesis/assembly of proteins that bring about this effect is not yet known.

We report the dynamics of photosystem stoichiometry adjustment during chloroplast acclimation to predominantly PSI-light or PSII-light conditions. Changes in PSII (Q_A and reaction center protein content) and PSI (P700 and PSI reaction center protein content) were monitored during this acclimation. We found coordinated changes between the structural and functional components for each of the photosystems (Q_A , D1, D2, and Cyt b_{559} [9 kD] for PSII; P700 and *psaA/psaB* gene products for PSI), leading to PSII:PSI ratio adjustments. We also present evidence that PSI and PSII are the signal perception light sensors, in which the photosystem accessory pigments act as the light-quality photoreceptor molecules.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Pisum sativum (pea) and *Hordeum vulgare* (barley) were grown in the laboratory at room temperature in the dark for 3 d, followed by continuous illumination for 7 to 8 d. The growth illumination was provided either by incandescent light bulbs filtered through red Plexiglas (PSI light; 25 μmol of photons $\text{m}^{-2} \text{s}^{-1}$) or by cool-white fluorescent lamps filtered through yellow Plexiglas (PSII light; 55 μmol of photons $\text{m}^{-2} \text{s}^{-1}$) (Glick et al., 1986). The relative intensity of the two light sources was set so that the integrated A of light by the chloroplast was about the same under such PSI-light and PSII-light conditions (Glick et al., 1986). At the end of the growth period, PSI-light-grown plants were switched to PSII light and vice versa. Nearly and fully expanded leaves were harvested, at the time indicated in specific experiments, after the light transition.

Synechococcus sp. PCC 7002 (*Agmenellum quadruplicatum*) cells were grown in the medium described by Arnon et al. (1974). The cyanobacteria cultures were bubbled with 3% CO_2 and stirred continuously to allow uniform growth conditions within each culture. Illumination was provided as described above. Cultures were grown at 30 to 34°C, and cells were harvested in the late log phase.

Thylakoid Membrane Isolation

Thylakoid membranes from pea and barley plants were isolated by grinding leaves (mortar and pestle) on ice in a buffer containing 400 mM Suc, 50 mM Tricine, 10 mM NaCl, 0.5% BSA, and 0.5% sodium ascorbate (pH 8.0). The homogenate was filtered through a 20- μm nylon mesh and centrifuged at 12,000g for 5 min at 4°C to yield thylakoids. The pellet was resuspended in approximately 1 mL of 125 mM Tris-HCl, 10 mM EDTA, 10% glycerol (pH 6.8) with the help of a microhomogenizer. The Chl ($a+b$) content of the thylakoid membrane suspension was determined in 80% acetone (Arnon, 1949). Thylakoid membranes from the Chl b -less barley mutant (chlorina f2) were prepared as described elsewhere (Glick and Melis, 1988). For SDS-PAGE and western blot analysis, thylakoid membranes were centrifuged in a Beckman microfuge for 5 min, and the pellet was resus-

pended in solubilization buffer containing 150 mM Tris-HCl, 4 M urea, 7% SDS, 20% glycerol, and 5% β -mercaptoethanol (pH 6.8) to give a final Chl ($a+b$) concentration of 1 mM. Samples were incubated at room temperature for 30 min to fully solubilize proteins and stored at -80°C .

Thylakoid membranes from the cyanobacterium *A. quadruplicatum* were isolated as follows. Cells were harvested by centrifugation at 8000g for 5 min at 4°C. Cells were washed once in hypotonic buffer containing 10 mM NaCl, 5 mM MgCl_2 , and 50 mM Tris-HCl (pH 7.8) and centrifuged again at 8000g for 5 min at 4°C. The pellet was resuspended in 40 mL of hypotonic buffer, and cells were disrupted by sonication (five times for 30 s each at continuous mode with a 1-min interval between sonications) in a Branson sonifier operated at a power setting of 8. Cell debris were removed by centrifugation at 11,000g for 10 min at 4°C. The supernatant was centrifuged at 40,000g for 60 min at 4°C. Thylakoid membranes were resuspended in hypotonic buffer. Chl content was measured in 80% acetone (Arnon, 1949).

Photosystem Quantitation

The PSI and PSII content was measured from the concentrations of P700 and Q_A , respectively, as described previously (Melis and Brown, 1980; Melis and Anderson, 1983). Quantitation of PSII in *A. quadruplicatum* was obtained from the concentration of the primary electron acceptor pheophytin, as described elsewhere (Melis et al., 1992). The reaction mixture contained approximately 10 μM Chl suspended in 20 mM Tris-HCl, 35 mM NaCl, 2 mM MnCl_2 , 2 μM methylviologen, 2 μM indigodisulfonate (pH 7.8), and sufficient sodium dithionite to lower the redox potential to -490 mV . Triton X-100 was added to the reaction mixture to 0.025% for wild-type or 0.05% for mutant samples.

Thylakoid Membrane Protein Analysis

Thylakoid membrane proteins were resolved by SDS-PAGE using the discontinuous buffer system of Laemmli (1970) with 15% acrylamide resolving gel and 4.5% acrylamide stacking gel. Gels were run either in the absence or presence of 1 M urea, as indicated in the figure legends. The gel lanes were loaded on the basis of Chl (5–20 nmol). Electrophoresis on $0.15 \times 14 \times 16\text{-cm}$ slab gels was performed at a constant current of 10 to 12 mA until the Chl pigments reached the anode end of the gel (18–20 h).

Immunoblot Analysis

Identification of reaction center polypeptides was accomplished with immunoblot analysis upon transfer of proteins to nitrocellulose and by using specific polyclonal antibodies raised in rabbits against the PSII reaction center D1/32-kD (*psbA* gene) and D2/34-kD (*psbD* gene) proteins. Similarly, polyclonal antibodies were raised in rabbits against the PSI reaction center heterodimer (*psaA/psaB* gene) and against the Chl $a-b$ LHC-II. The cross-reaction of proteins with these antibodies was used to quantitate the level of the respective photosystem and antenna apoproteins in thylakoids as a function of time during chloroplast acclimation to a particular

light regimen. Electrophoretic transfer of the SDS-PAGE-resolved polypeptides to nitrocellulose and the subsequent incubations with the antibodies and with alkaline phosphate-conjugated secondary antibodies were performed as described previously (Smith et al., 1990). Cross-reaction was quantitated by scanning the nitrocellulose membranes with an LKB-Pharmacia XL laser densitometer. The width of the scanning beam in the densitometer was set at 4 mm to account for most of the bandwidth in the immunoblots. This approach minimized variations in apparent A that might have resulted because of nonuniformity of the bands in the immunoblots.

RESULTS

Photosystem Ratio Adjustment during Chloroplast Acclimation to PSI or PSII light

Changes in the photosystem composition of thylakoids are a long-term but fully reversible response. Figure 1 shows the kinetics by which changes in the P700:Chl, Q_A :Chl, and PSII:PSI (Q_A :P700) ratios occur. In this experimentation, pea

plants were first acclimated to a PSI-light environment. Under these growth conditions, pea thylakoids had the following ratios (mmol mol^{-1}): P700:Chl, 1.1 (Fig. 1A); Q_A :Chl, 2.8 (Fig. 1B); and PSII:PSI, 2.5:1 (Fig. 1C). At zero time, the pea plants were switched to PSII-light conditions (Fig. 1, left panels). Quantitation of P700 (PSI) and Q_A (PSII) relative to total Chl showed a gradual increase in P700 content (Fig. 1A, left panel) and a concomitant decrease in Q_A content (Fig. 1B, left panel). In consequence, the PSII:PSI ratio (2.5:1) of nearly expanded leaves decreased as a function of time in PSII light (Fig. 1C, left panel) until it reached a new steady-state value of about 1.25:1 with a half-time of about 20 h.

Upon transfer of the pea plants back to PSI-light conditions (Fig. 1, right panels), the change in the above parameters was reversed such that the PSII:PSI ratio gradually increased to the value typical for PSI-light-grown plants (Glick et al., 1987). A similar dynamic response was observed earlier with cyanobacteria (Allen et al., 1989). It is of interest to investigate the mechanics of this acclimation, both at the functional (Q_A , P700) and structural (protein content) levels. To this end, we isolated thylakoids and measured the concentration of reaction center proteins during a PSI-light \rightarrow PSII-light acclimation and vice versa.

PSI-Light \rightarrow PSII-Light Transition

As shown in Figure 1, a PSI-light \rightarrow PSII-light transition caused a prompt change in the PSII:PSI ratio of pea chloroplast (half-time = 20 h, initial PSII:PSI = 2.5, final PSII:PSI = 1.25). Changes in the reaction center protein content under these experimental conditions were investigated. Samples were harvested at 0, 2, 8, and 24 h after pea plants were transferred from PSI-light to PSII-light conditions. Thylakoid membranes were isolated from each sample, and proteins were resolved by SDS-PAGE. Proteins were transferred to nitrocellulose and then probed with polyclonal antibodies raised against the PSI reaction center polypeptides. Under these conditions, SDS-PAGE and immunoblot analysis showed an increase in the PSI reaction center protein content (Fig. 2A). The laser-densitometric analysis showed that the level of PSI reaction center protein (*psaA/psaB* gene products) increased by about 80% during the initial 24-h period following transition to PSII light (Fig. 2B). Comparison of the kinetics in Figure 2B with those in Figure 1A (left panel) suggested that PSI-protein accumulation precedes that of functional P700 in thylakoids. The results suggest that a PSI-light \rightarrow PSII-light transition induces a prompt increase in the rate of PSI-protein accumulation.

Additional SDS-PAGE and immunoblot analysis with specific polyclonal antibodies raised against the PSII reaction center proteins revealed a decrease in the concentration of both D1 and D2 proteins, occurring essentially in accordance with the Q_A content in the acclimating thylakoids (Fig. 3A). Changes in the concentration of both D1 and D2 reaction center proteins showed two kinetic phases: a rapid decrease during the first 2-h period and a slower decrease during the 2- to 24-h period after PSI-light \rightarrow PSII-light transition (Fig. 3B). We consistently observed different amplitudes in the decrease of the D1 and D2 reaction center polypeptides (Fig. 3B). The level of D1 protein decreased by about 30% of its

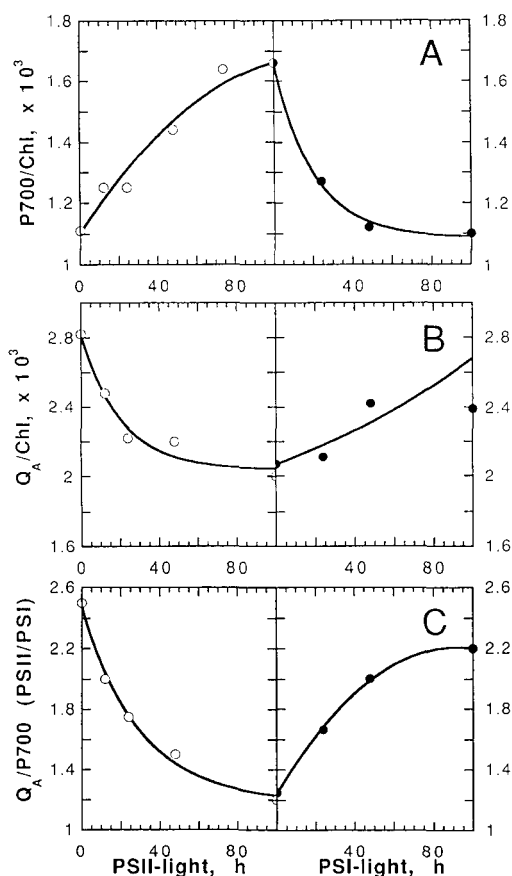


Figure 1. Changes in the ratios P700:Chl (A), Q_A :Chl (B), and PSII:PSI (C) in response to changes in the light quality during plant growth. Pea plants were first acclimated to PSI-light. They were switched (at time zero) to a PSII-light environment (left panels). After about 100 h, the same plants were switched back to a PSI-light environment (right panels).

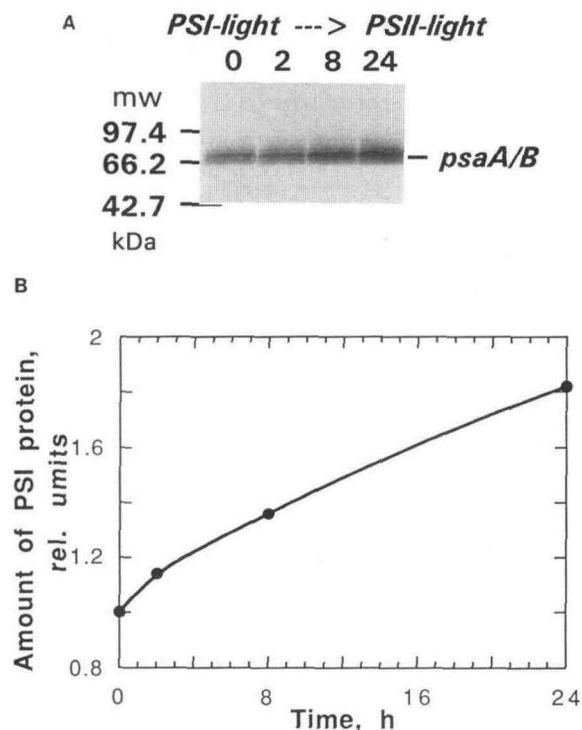


Figure 2. Changes in the concentration of PSI reaction center proteins following a PSI-light \rightarrow PSII-light transition. A, Immunoblot analysis of thylakoid membrane proteins from pea plants acclimating after a PSI-light \rightarrow PSII-light transition. Samples were harvested at 0, 2, 8, and 24 h after transition to PSII light, and thylakoid membranes were isolated from each sample as described in "Materials and Methods." Lanes were loaded with 20 nmol of Chl (a+b). Thylakoid proteins were resolved by SDS-PAGE and then transferred to nitrocellulose. Levels of the PSI reaction center proteins were estimated from the cross-reaction with specific polyclonal antibodies. B, Laser-densitometric analysis of the cross-reactions shown in A. Note the approximately 80% increase in the level of the PSI reaction center proteins during the 0- to 24-h acclimation period.

initial value during the first 2-h period and by about 35% of its initial value during the 24-h period after transition to PSII light. However, the level of the D2 protein showed only about 14% decrease during the 24-h period. The results suggested a faster rate in the decrease of the D1 relative to the D2 protein (Fig. 3B). This may reflect enhanced D1 degradation under the predominantly PSII-light conditions. The different response of the D1 and D2 proteins to the PSI-light \rightarrow PSII-light transition suggested different control mechanisms for the regulation of degradation of the two proteins.

PSII-Light \rightarrow PSI-Light Transition

A transition of pea plants from PSII-light to PSI-light conditions caused a reversal of the earlier change in the PSII:PSI ratio (Fig. 1, right panel; half-time = 30 h, initial PSII:PSI = 1.25, final PSII:PSI = 2.2). To study further the changes occurring following a PSII-light \rightarrow PSI-light transition, the reaction center protein level was measured. Samples

were harvested 0, 2, 8, and 24 h after transition to PSI-light conditions, and thylakoid membranes were isolated from each sample. Total thylakoid proteins were resolved by SDS-PAGE, followed by immunoblot analysis. Under these conditions, analysis revealed a decrease in the PSI reaction center protein content (Fig. 4A). The PSI reaction center protein content was decreased to about 84% of its initial value during the first 2-h period and to about 60% of the initial value during the 24-h period after transition to PSI-light conditions (Fig. 4B). This occurred essentially in concert with the declining P700 content of thylakoids (Fig. 1A, right panel).

Changes in the concentration of PSII reaction center proteins were measured by SDS-PAGE, followed by immunoblot analysis with specific polyclonal antibodies. A gradual increase in the concentration of the D1 and D2 proteins was observed following the PSII-light \rightarrow PSI-light transition (Fig. 5A). The laser-densitometric analysis showed a relatively rapid increase during the first 2-h period (approximately 23%

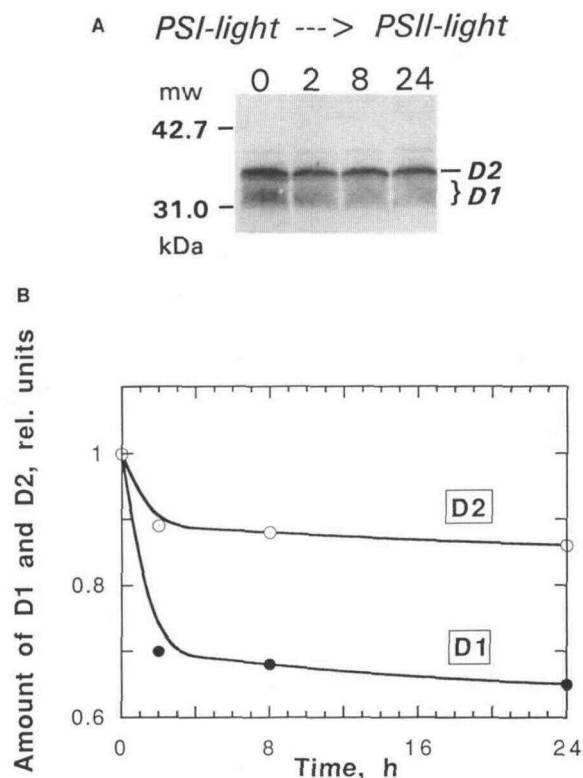


Figure 3. Changes in the concentration of PSII reaction center proteins (D1 and D2) following a PSI-light \rightarrow PSII-light transition. A, Immunoblot analysis of pea thylakoids acclimating (0-24 h) after a PSI-light \rightarrow PSII-light transition. Steady-state levels of PSII reaction center proteins were measured from the cross-reaction with specific polyclonal antibodies. For other conditions, see legend of Figure 2. B, Changes in the level of PSII reaction center protein D1 (●) and D2 (○) as a function of time. The intensity of the cross-reaction between antibody and PSII reaction center protein (D1 and D2) was determined by laser-densitometric analysis. Note the approximately 14% decrease in the steady-state level of D2 and the approximately 35% decrease in the level of the D1 protein during the 0- to 24-h period.

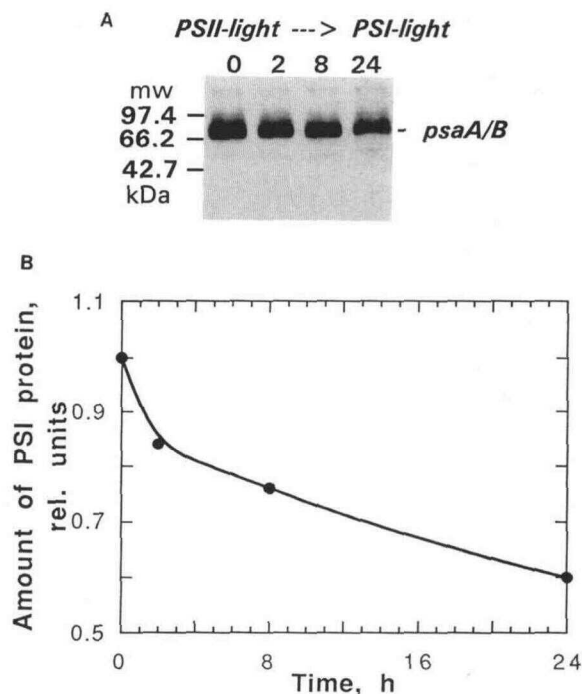


Figure 4. Changes in the concentration of PSI reaction center proteins following a PSII-light \rightarrow PSI-light transition. A, Immunoblot analysis of pea thylakoids acclimating after a PSII-light \rightarrow PSI-light transition. The gel contained 1 M urea. For other conditions, see legend of Figure 2. B, The level of PSI reaction center proteins was measured from the cross-reaction with specific polyclonal antibodies by laser-densitometric analysis. The level of PSI decreased to about 60% of the initial amount during the 0- to 24-h period.

increase of the initial value) and a slower increase during the 24-h period (approximately 50% increase from the initial value) (Fig. 5B). The prompt increase in the PSII reaction center protein content occurred with somewhat faster kinetics than that of Q_A in the acclimating thylakoids (Fig. 1B, right panel).

The D1 reaction center protein of PSII is known for its frequent turnover in the chloroplast (Mattoo and Edelman, 1987). A primary membrane-bound degradation product of D1 protein was identified as a 23.5-kD polypeptide that derived from the amino-terminal portion of the D1 protein (Greenberg et al., 1987). Under our SDS-PAGE conditions, this protein fragment migrated to about 20 kD (Fig. 6A). Using immunoblot analysis, we quantitated the steady-state level of this D1 degradation product following a PSII-light \rightarrow PSI-light transition. The steady-state level of the primary degradation product decreased to approximately 70% of its initial value during the 24-h period after a PSII-light \rightarrow PSI-light transition (Fig. 6B).

In agreement with the earlier results, the level of the 9-kD Cyt b_{559} apoprotein increased under these conditions (Fig. 6A). Laser-densitometric analysis showed about 50% increase in the concentration of Cyt b_{559} apoprotein during the 24-h period after transition to PSI-light conditions. This measurement corroborates the increase in PSII protein content, measured by the amount of D1 and D2 under these conditions (Fig. 5B). The results show that the level of PSII reaction center proteins increased approximately 50% during the 24-h period.

transition, the concentration of the PSII reaction center proteins increases by about 50% during a 24-h period and, at the same time, the rate of D1 turnover is decreased, suggesting a greater stability of the PSII reaction center proteins under the PSI-light regimen.

Changes in the LHC-II Content in Response to Light-Quality Transitions

The above results show that changes in the light regimen during plant growth (PSI light \rightarrow PSII light and vice versa) induce adjustments in the photosystem stoichiometry in a well-coordinated manner. To investigate whether changes in the photosystem composition of thylakoids are accompanied by changes in the auxiliary Chl $a-b$ light-harvesting antenna, the relative Chl b content and the level of LHC-II apoproteins were measured during a PSI-light \rightarrow PSII-light transition and vice versa.

Changes in the relative Chl b content in pea thylakoids were measured after the PSI-light \rightarrow PSII-light transition and vice versa (Fig. 7A). The relative Chl b content was decreased from 0.24 to 0.21 during the 24-h period following the PSI-light \rightarrow PSII-light transition. Conversely, following a PSII-light \rightarrow PSI-light transition, the relative Chl b content was

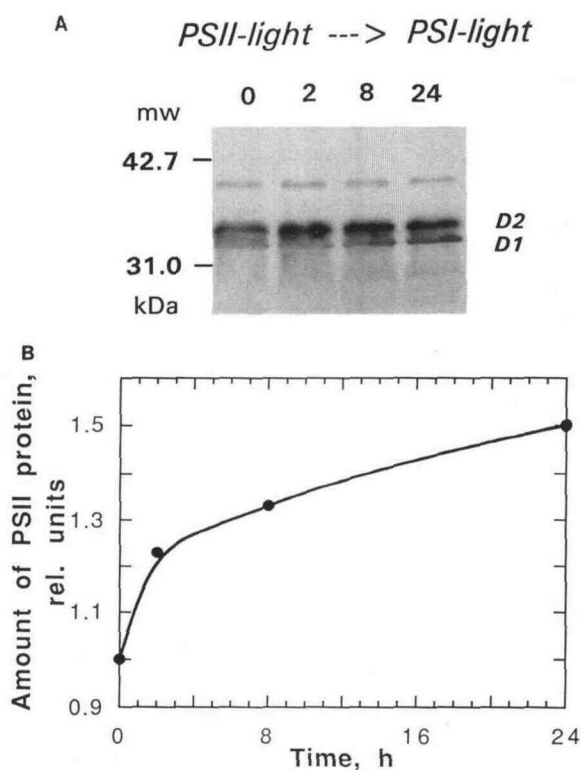


Figure 5. Changes in the concentration of PSII reaction center proteins (D1 and D2) following a PSII-light \rightarrow PSI-light transition. A, Immunoblot analysis of pea thylakoids acclimating (0-24 h) after the PSII-light \rightarrow PSI-light transition. The gel contained 1 M urea. For other conditions, see legend of Figure 2. B, The concentration of the PSII reaction center proteins was measured from the cross-reaction with specific polyclonal antibodies by laser-densitometric analysis. The level of PSII reaction center proteins increased approximately 50% during the 24-h period.

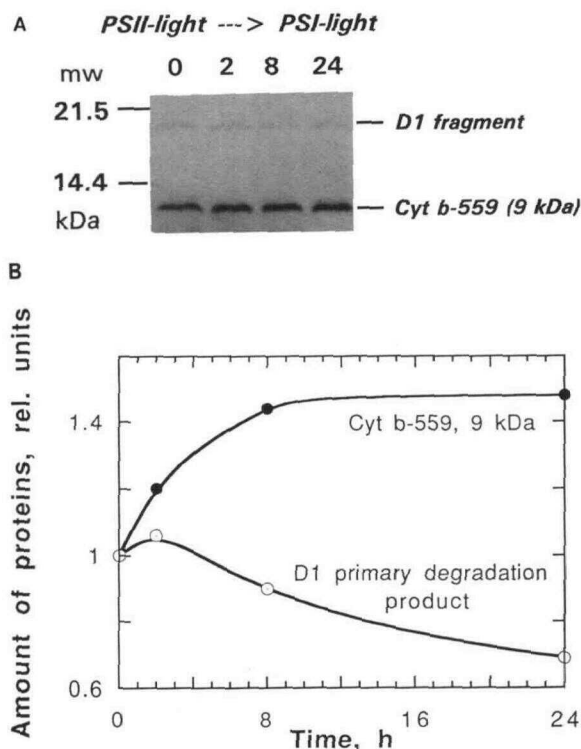


Figure 6. Changes in the steady-state amount of the D1 primary proteolysis fragment and in the level of the Cyt *b*₅₅₉ (9 kDa) protein in pea thylakoids following a PSII-light → PSI-light transition. **A**, Immunoblot analysis of pea thylakoids acclimating (0–24 h) after the PSII-light → PSI-light transition. The gel contained 1 M urea. Steady-state levels of the Cyt *b*₅₅₉ (9 kDa) and of the membrane-bound D1 protein degradation product were measured from the intensity of the cross-reaction with specific polyclonal antibodies. **B**, Laser-densitometric analysis of the concentration of the Cyt *b*₅₅₉ (9 kDa) (●) showed an increase by about 50% of its initial value in the 0- to 24-h period. The steady-state level of the membrane-bound partial proteolysis product of D1 (○) was decreased to approximately 70% of its initial value during the 24-h acclimation period.

increased to the value of PSI-light-grown pea thylakoid (from 0.22–0.24) during a 24-h period. These results suggested changes in the level of LHC-II apoproteins during these light-quality transitions.

Thylakoid membranes were isolated from acclimating pea plants, and SDS-PAGE and immunoblot analysis with specific anti-LHC-II antibodies were performed as described above. The intensity of cross-reactions between antibody and the subunits of the LHC-II were measured by laser densitometry. Figure 7B shows a gradually declining level of LHC-II apoprotein following a PSI-light → PSII-light transition, occurring in accordance with the relative Chl *b* content of thylakoids (Fig. 7A). Twenty-four hours after the PSI-light → PSII-light transition, the amount of LHC-II was decreased to approximately 85% of the initial value. However, the level of LHC-II increased upon transition of plants from PSII-light to PSI-light conditions (approximately 25% increase compared with the initial value). Changes in LHC-II apoprotein

content (Fig. 7B) were parallel with those reported for the relative Chl *b* content (Fig. 7A) and also parallel with those of PSII content in the acclimating thylakoids. Significantly, partial degradation products of LHC-II, which were reported to occur under irradiance-stress conditions (Melis, 1992), were not observed during these light transitions (data not shown).

In summary, these results suggest that photosystem stoichiometry adjustments, when induced by light-quality changes, proceed in a well-coordinated manner in the chloroplast and entail significant alterations in the photosystem composition and structure of thylakoids. Undoubtedly, light quality must influence the biosynthesis/assembly of thylakoid membrane proteins without causing undue stresses in the chloroplast. In support of this contention is the apparent

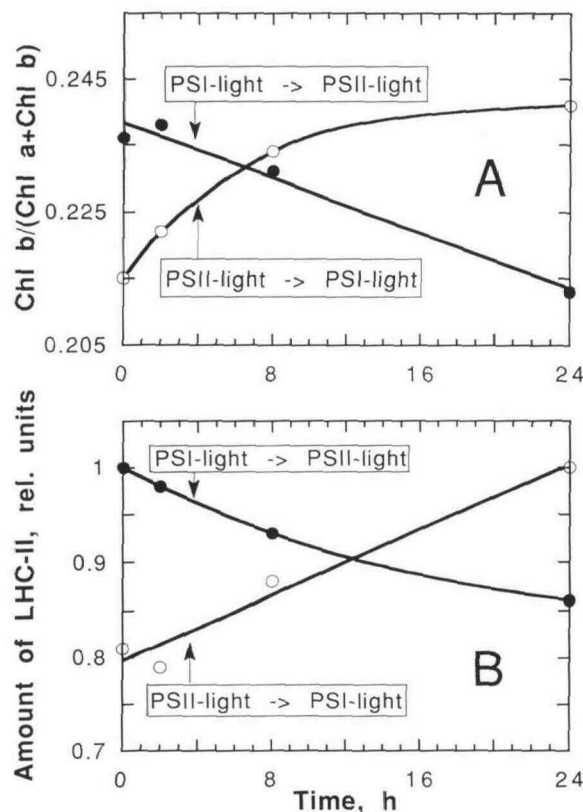


Figure 7. Changes in the level of Chl *b* and LHC-II content in pea thylakoids following a PSI-light → PSII-light transition (●) and vice versa (○). **A**, The relative Chl *b* content decreased after the PSII-light → PSI-light transition and, conversely, increased to the value of PSI-light-grown pea thylakoid after the PSII-light → PSI-light transition. SD in these measurements was ± 0.002. **B**, Changes in the LHC-II apoprotein content following light-quality transitions. Lanes were loaded with 10 nmol of Chl (a+b). In this quantitation, the amount of all subunits associated with the LHC-II was estimated from the cross-reaction with specific antibodies by laser-densitometric analysis. The level of LHC-II shows a featureless decrease after the PSI-light → PSII-light transition (●), and a monotonous increase after the PSII-light → PSI-light transition (○). These changes are parallel with the changes in the Chl *b* content as measured in A.

lack of accumulation of precursors, modified forms, or partial degradation products of the photosystem constituent proteins.

Chloroplast Perception of Light Quality

It was proposed that accessory photosynthetic pigments serve as photoreceptors and thus mediate light-quality-dependent changes in photosystem stoichiometry (Melis, 1991). This premise was investigated directly by utilizing the Chl *b*-less chlorina f2 mutant of barley (*H. vulgare*) and the PBS-less mutant of the cyanobacterium *A. quadruplicatum* (*Synechococcus* PCC 7002).

Wild-type barley showed a PSII:PSI ratio of 1.9:1 when it was grown under direct sunlight. However, the Chl *b*-less chlorina f2 mutant of barley showed a higher PSII:PSI ratio, about 3:1, under the same conditions (Table I). Wild-type *A. quadruplicatum* had a PSII:PSI ratio of 0.5:1 when grown under white light. The PBS-less mutant had a PSII:PSI ratio of about 1:1 under the same light conditions (Table I). Measurements of the functional light-harvesting antenna size showed that absence of Chl *b* in the chlorina f2 mutant resulted in a smaller antenna size, from 250 Chl (*a+b*) to about 50 Chl *a* molecules in PSII and from 220 Chl (*a+b*) to about 150 Chl *a* in PSI (Glick and Melis, 1988). Similarly, the absence of PBS in the *A. quadruplicatum* resulted in a severe attenuation of the light-harvesting capacity of PSII. We suggest that an elevated PSII:PSI ratio in the mutants is a response of the photosynthetic apparatus to the altered absorption/utilization of light by the two photoreactions. This adjustment is a meaningful response because it restores the balance of light absorption between PSII and PSI, in essence correcting the effect of the mutation.

Furthermore, wild-type barley and *A. quadruplicatum* both showed the anticipated PSII:PSI ratio adjustment in response to the quality of illumination (Table I). However, in the absence of accessory pigments (Chl *b* and phycobilins), the photosystem ratio of the mutants could not be influenced by the quality of illumination. The PSII:PSI ratio was about 3:1 for the chlorina f2 mutant and about 1:1 for the PBS-less mutant, irrespective of the light regimen during growth (PSI light or PSII light, Table I). Experimental analysis revealed that changes in light quality (PSI light → PSII light and vice versa) initially cause imbalance in light absorption and rate of electron transport between the two photosystems in the wild type. This imbalance is rectified by the adjustment of the PSII:PSI ratio. Changes in light quality, however, do not affect the distribution of excitation energy to the two photosystems in the mutants. Thus, the evidence suggests a cause-and-effect relationship between the initial distribution of excitation energy between the two photosystems and the ultimate PSII:PSI ratio in thylakoids.

DISCUSSION

The adjustment of the photosystem stoichiometry to different light qualities is a long-term response when compared with the so-called "state transitions" (Wang and Myers, 1974) and with the underlying phosphorylation and dephosphorylation of the LHC-II through a redox-regulated kinase (Bennett et al., 1980; Allen et al., 1981). The latter phenomena bring about organizational changes in the thylakoid membrane, they occur on the order of minutes (versus hours for the photosystem stoichiometry adjustment), and they do not involve changes in the composition of the thylakoid mem-

Table I. Signal perception for photosystem stoichiometry adjustment in chloroplasts

Pigment (Chl *a* and Chl *b*) and photosystem quantitation was obtained spectrophotometrically. Quantitation of PSII and PSI (mol:mol) was obtained from spectrophotometric measurements of Q_A or pheophytin photoreduction (PSII) and P700 photooxidation (PSI). Note the adjustment of the PSII:PSI ratio to light quality in wild type and the lack of response in the mutants.

System	Chl <i>a</i> :Chl <i>b</i>	$A_{625}:A_{678}$	Chl:PSI	Chl:PSII	PSII:PSI
<i>H. vulgare</i> (barley) chloroplast					
Wild type					
PSII-light	3.5:1		500:1	350:1	1.4:1
Sunlight	2.9:1		595:1	315:1	1.9:1
PSI-light	2.6:1		725:1	270:1	2.7:1
Chl <i>b</i> -less chlorina f2					
PSII light			315:1	118:1	2.7:1
Sunlight			315:1	105:1	3.0:1
PSI light			325:1	115:1	3.1:1
<i>A. quadruplicatum</i> strain PR-6 cells					
Wild type					
PSII light		1.0:1	120:1	370:1	0.32:1
White light		1.3:1	125:1	250:1	0.50:1
PSI light		1.7:1	135:1	235:1	0.57:1
PBS-less PR-6					
PSII light		0.24:1	175:1	160:1	1.1:1
White light		0.24:1	170:1	165:1	1.0:1
PSI light ^a					

^a We were unable to grow healthy cell cultures under these conditions.

brane (Haworth et al., 1982; Fork and Satoh, 1986; Ranjeva and Boudet, 1987).

Changes in the quality of illumination during plant growth bring about adjustments in the photosystem composition (PSII:PSI ratio) in the thylakoid membrane. In this paper, we reported the kinetics of change in the functional cofactors of the photosystems (P700 and Q_A), in the level of PSI and PSII reaction center proteins, and in the level of LHC-II following a PSI-light \rightarrow PSII-light transition and vice versa. Results from this study (spectrophotometric and immunochemical) are reported on the basis of total Chl ($a+b$) in the samples. We used total Chl as a convenient point of reference and to simplify the analysis. When reporting component ratios (e.g. Q_A :P700, PSII protein:PSI protein), the Chl content cancels out of the ratio. Therefore, these ratios provide a true measure of the relative concentration of the two photosystems in pea leaves. This approach, however, will not provide information concerning the absolute change of PSII and PSI on a per plastid basis. There are conflicting reports in the literature about whether a photosystem ratio adjustment is mediated solely by absolute changes in the amount of PSI (Glick et al., 1986; Fujita and Murakami, 1987) or in the amount of PSII per chloroplast (Anderson, 1986). It appears that a different experimental approach is needed to measure absolute rates of PSII and PSI biosynthesis under PSII-light or PSI-light conditions.

The relative concentration of reaction-center proteins showed a prompt change in the initial 2-h period followed by slower changes during the 24-h period after a transition in the light quality. It is of interest to observe that, following a PSI-light \rightarrow PSII-light transition, PSI protein accumulation (Fig. 2) occurred faster than the P700 concentration increase (Fig. 1A, left panel). Similarly, following a PSII-light \rightarrow PSI-light transition, PSII protein accumulation (Fig. 5) occurred faster than the Q_A concentration increase (Fig. 1B, right panel). In the down-regulation of photosystem components, however, changes in the relative concentration of the functional cofactors of PSI (P700) and PSII (Q_A) paralleled those of the reaction center proteins (Fig. 1A, right panel, and Fig. 4; Fig. 1B, left panel, and Fig. 3) (Aizawa et al., 1992). Increased levels of PSI (P700, PSI reaction center proteins) or decreased levels of PSII (Q_A , PSII reaction center proteins) and of LHC-II upon a PSI-light \rightarrow PSII-light transition were fully reversible upon reversal of the light-quality regimen. These results strengthen the evidence for the existence of a mechanism in photosynthetic organisms designed to sense and correct long-term imbalance in light absorption through adjustment and optimization of the PSII:PSI ratio (Melis, 1991). From the evolutionary point of view, it would appear that photosynthetic organisms possessing such acclimation mechanisms might enjoy a significant selective advantage over others with a fixed photosystem ratio in their thylakoid membranes. This advantage emanates both from an improved quantum efficiency of photosynthesis (Murakami and Fujita, 1988; Melis et al., 1989; Chow et al., 1990) and from the ensuing conservation of resources. The latter involves a cellular conservation of metabolic energy and of nutrient resources simply by directing biosynthetic activity only toward those components that are important to sustain an efficient photosynthesis.

The turnover rate of the reaction center D1 protein of PSII depends on the intensity of light absorbed by PSII (J.H. Kim and A. Melis, unpublished data). Thus, under PSII-light conditions, the rate of D1 turnover is faster; however, upon transfer to PSI light, the rate of D1 turnover becomes slower. These results show that, under PSI-light conditions, both the concentration and the stability of PSII reaction center proteins increase.

Our results showed that adjustments in the photosystem stoichiometry in the thylakoid membrane, occurring in response to changes in the light quality during plant growth, involve concerted changes in the Q_A , D1, D2, and Cyt b_{559} (9 kD) protein content (PSII), as well as in the P700 and *psaA/psaB* gene product content (PSI). The acclimation occurs in a well-coordinated manner in chloroplasts: accumulation of damaged PSII units (Kyle et al., 1984; Ohad et al., 1984; Powles, 1984; Demeter et al., 1987) or the appearance of a modified form of D1 (Callahan et al., 1990; Kettunen et al., 1991; Melis, 1992), which are observed under adverse irradiance conditions, were not observed during photosystem stoichiometry adjustment following light-quality transitions. Similarly, the level of the LHC-II (Chl *b* and LHC-II apoproteins) decreased under PSII-light conditions and increased under PSI-light conditions. These changes in the level of LHC-II are parallel with the changes in the concentration of PSII observed under the same conditions. Partial degradation products of LHC-II proteins, which appeared under irradiance-stress conditions (Melis, 1992), were not observed following light-quality transitions. These results again suggest an orderly response of the photosynthetic apparatus to sub-optimal light qualities as manifested by the gradual change in the photosystem composition of thylakoid membranes.

The results from this study further show that signal perception for photosystem stoichiometry adjustment occurs at the thylakoid membrane level as differential sensitization of pigments associated with PSII and PSI. The signal transduction pathway is probably activated by imbalance in the rate of electron flow between the two photosystems (Fujita et al., 1987), the determining factor in the signal transduction pathway being the redox state of intermediates such as the plastoquinone pool and/or the Cyt *b-f* complex.

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